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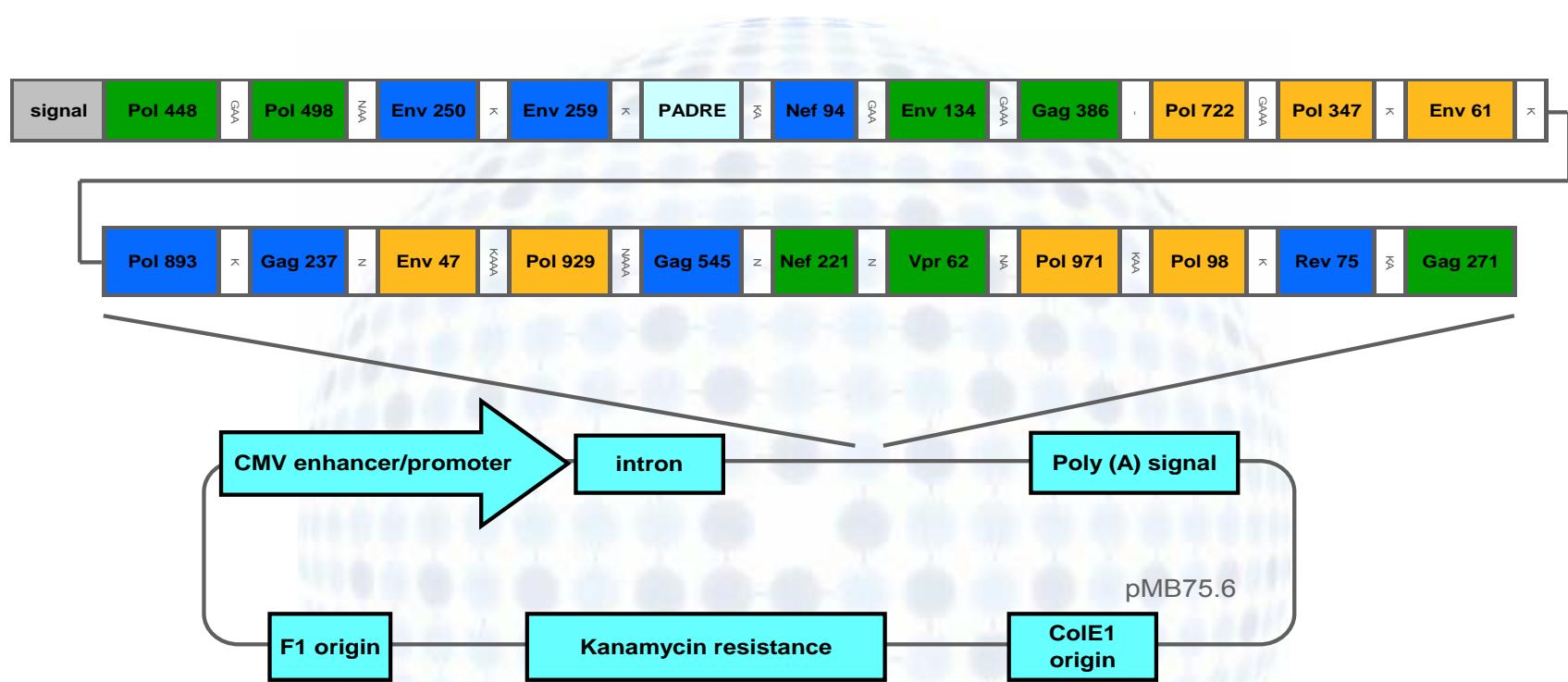
Use of HLA Transgenic Mice to Directly Assess Vaccine Immunogenicity



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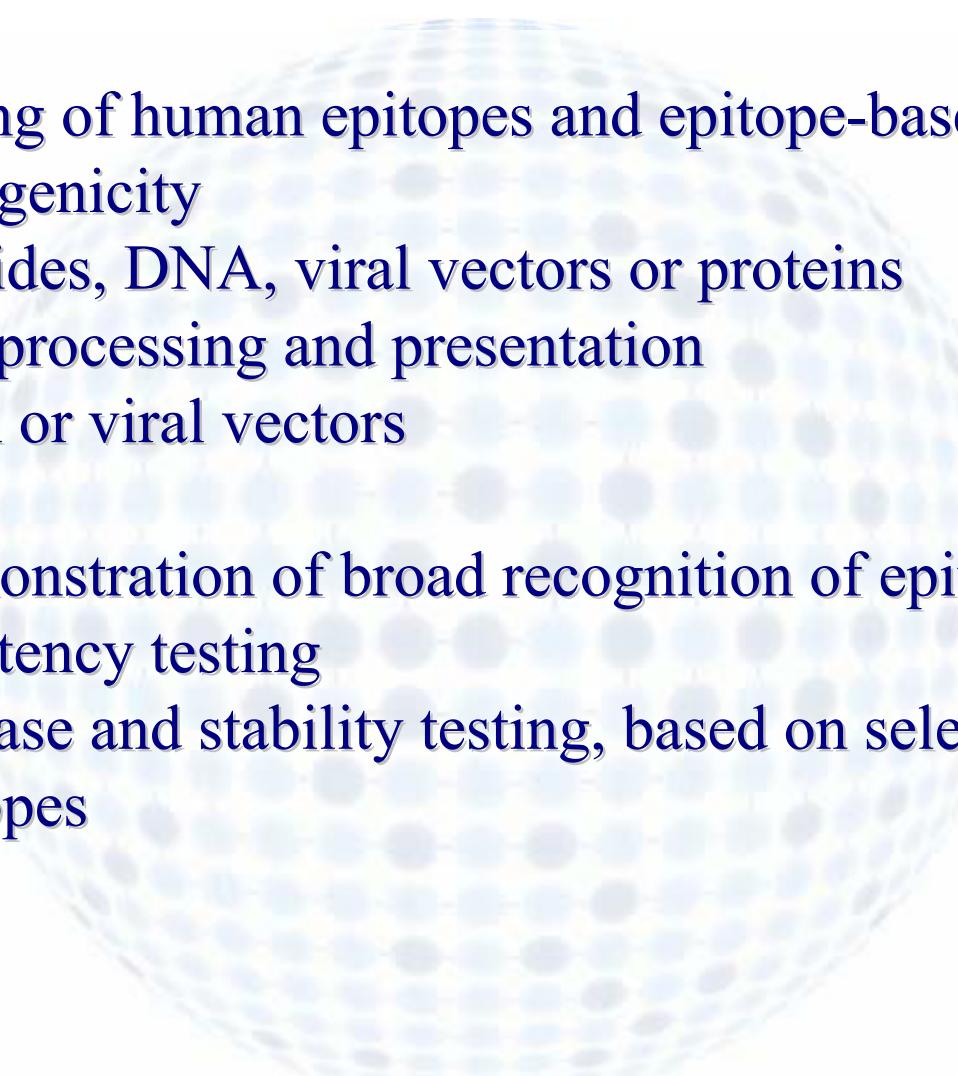
Epitope-Based HIV-1 Vaccines

Therapeutic Vaccine: EP HIV-1090



Encodes 21 HIV-derived CTL epitopes and PADRE
optimized for proteosomal processing

Why Use HLA Transgenic Mice?



- *In vivo* testing of human epitopes and epitope-based vaccines
 - Immunogenicity
 - Peptides, DNA, viral vectors or proteins
 - Epitope processing and presentation
 - DNA or viral vectors
 - R & D
 - Demonstration of broad recognition of epitopes
 - GLP potency testing
 - Release and stability testing, based on selected marker epitopes

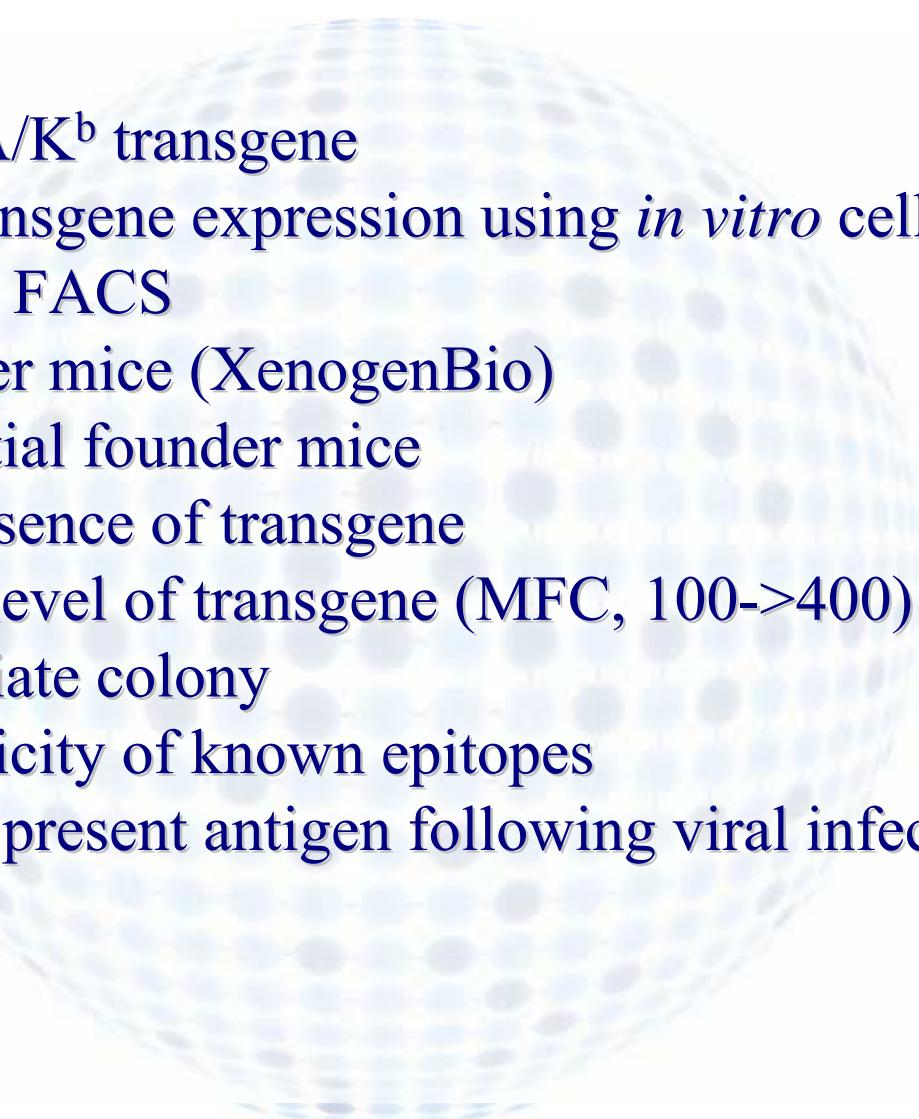
HLA Transgenic Mouse Colonies

Supertype	In-House Assays	Predicted Phenotypic Frequency (%)				
		Asian	Black	Euro.	N. Amer.	Average
A2	A*0201-6, A*6802 A*0301, A*1101	42.7	40.5	50.0	51.1	46.1
A3	A*31012, A*3301 B*0702, B*3501-3	56.7	51.6	48.0	47.8	51.0
B7	B*5301, B*5401 A*0101, A*3002	43.5	55.1	51.5	52.8	50.7
A1	A*2601, A*3201 A*2301, A*2402	18.7	54.8	53.9	52.0	44.8
A24	A*3002 B*1801, B*4001-2	49.6	21.7	19.4	19.7	27.6
B44	B*4402, B*44031	45.1	43.8	52.2	49.1	49.3
A1 + A2 + A3 + A24 + B7 + B44		~100	~100	~100	~100	~100

HLA Transgenic Mouse Colonies

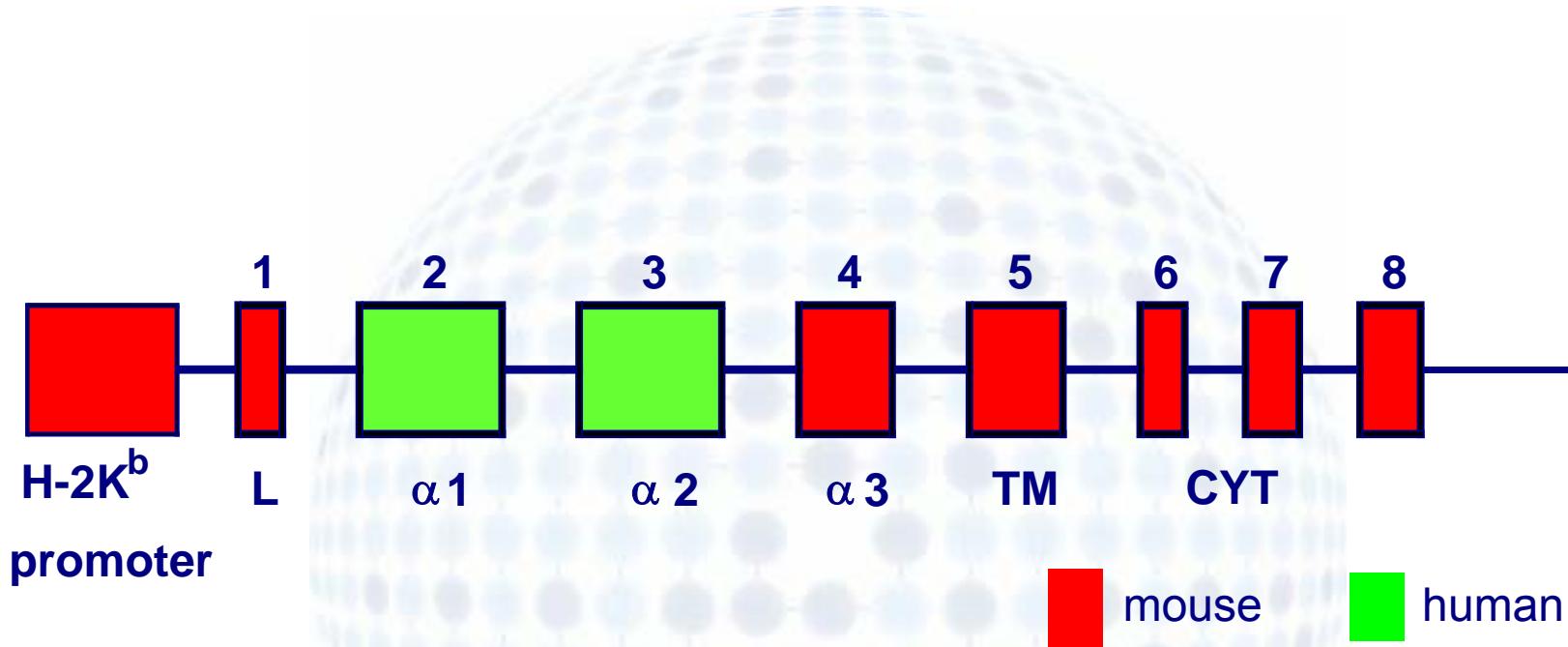
Supertype	Representative In-House Assays	Predicted Phenotypic Frequency (%)				
		Asian	Black	Euro. Cauc.	N. Amer. Cauc.	Average
DR1	B1*0101, B1*0401					
	B1*0404-5, B1*0701					
	B1*1101, B1*1302	88.2	93.8	93.2	94.9	92.5
	B1*0901, B1*1501					
	B1*0802					
DR3	B1*0301	5.2	22.4	24.7	21.0	18.3
DR5w12	DRB1*1201	15.3	9.6	3.4	2.2	7.6
DR1 + DR3 + DR5w12		94.3	99.4	98.7	98.9	97.8

Generation of HLA Transgenic Mice

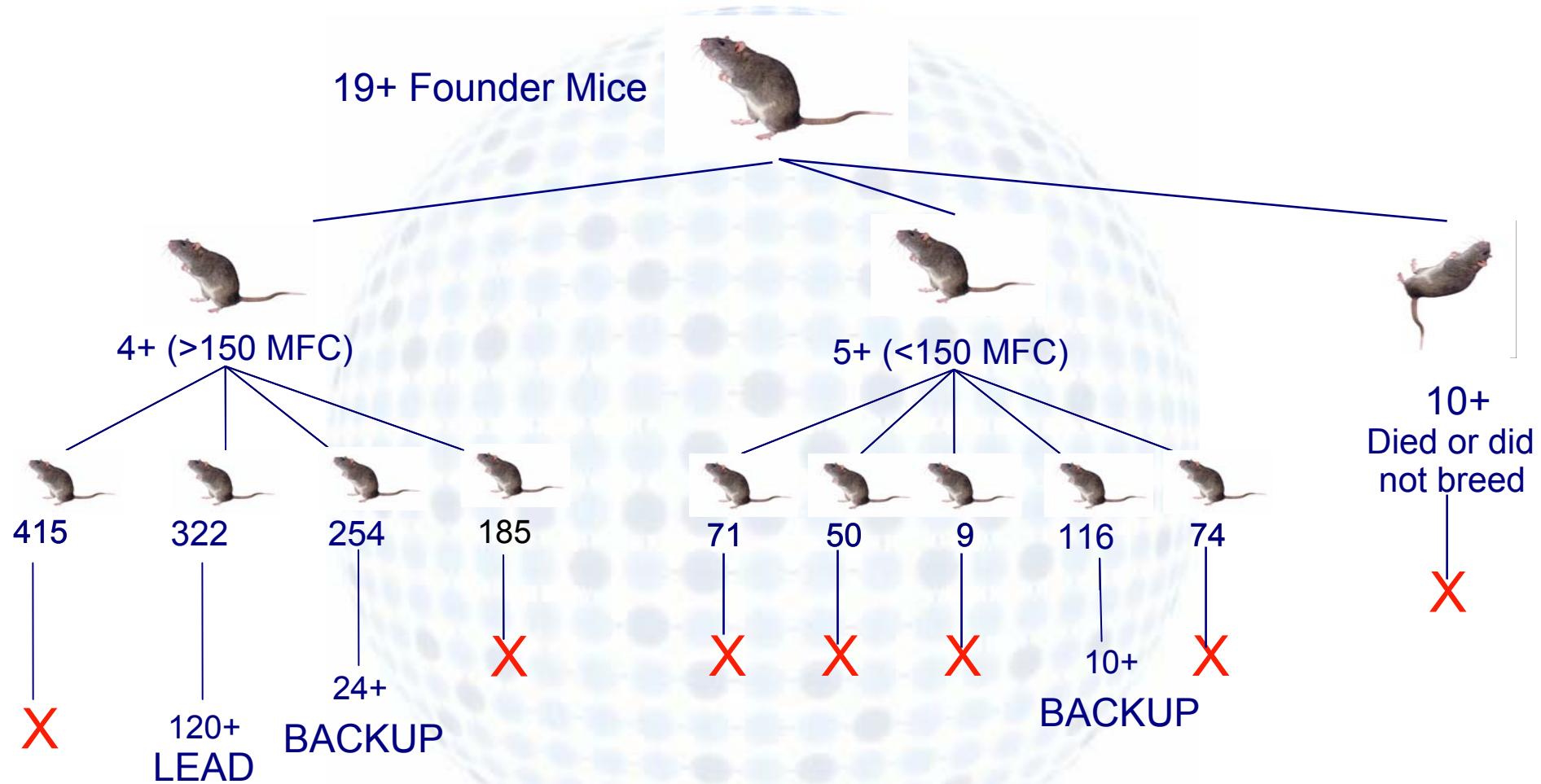


- Synthesize HLA/K^b transgene
- Demonstrate transgene expression using *in vitro* cell transfection and FACS
- Generate founder mice (XenogenBio)
- Evaluate potential founder mice
 - PCR for presence of transgene
 - Expression level of transgene (MFC, 100->400)
- Breeding to initiate colony
 - Immunogenicity of known epitopes
 - Process and present antigen following viral infection

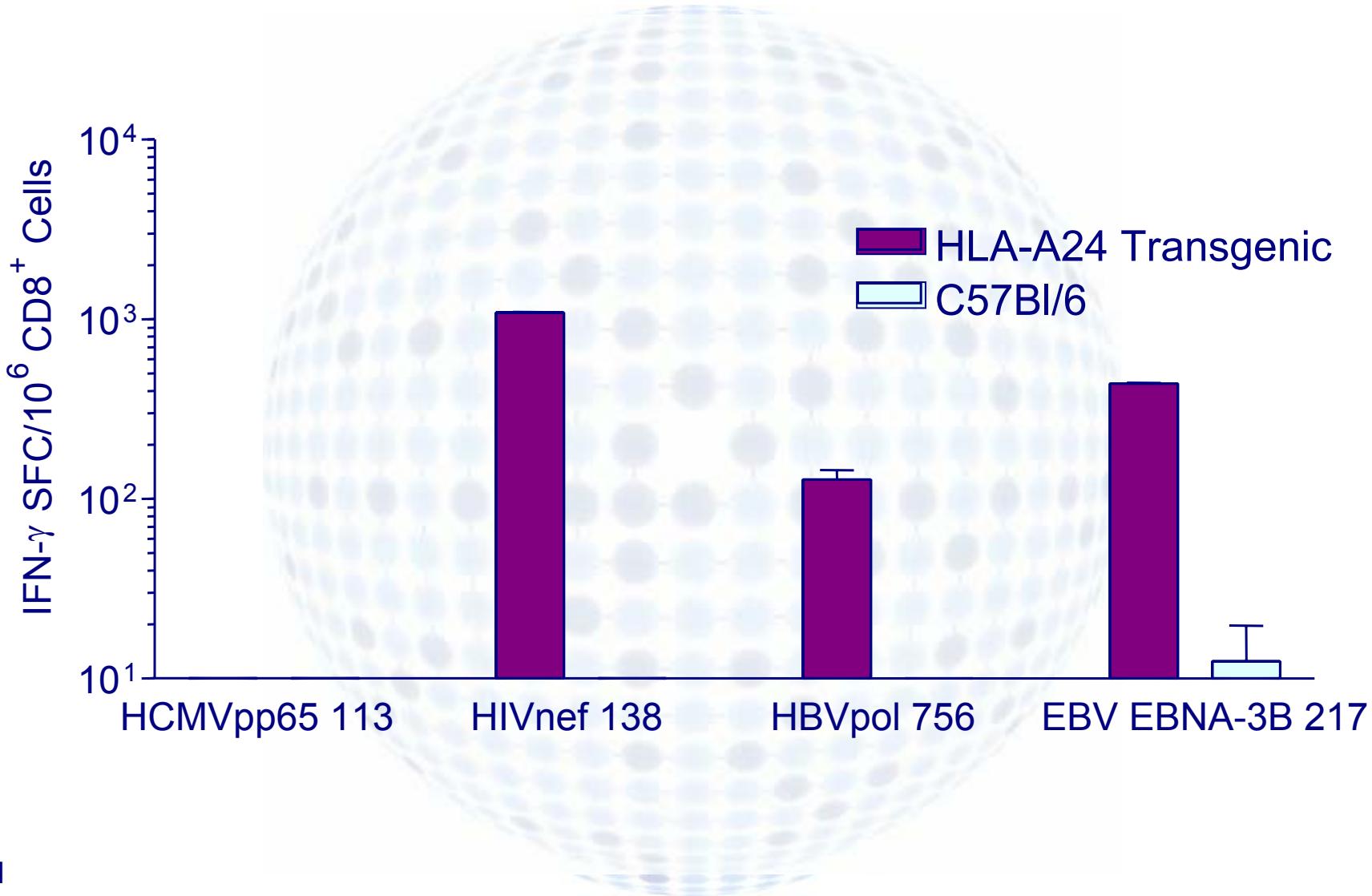
Structure of HLA-A and -B Transgenes



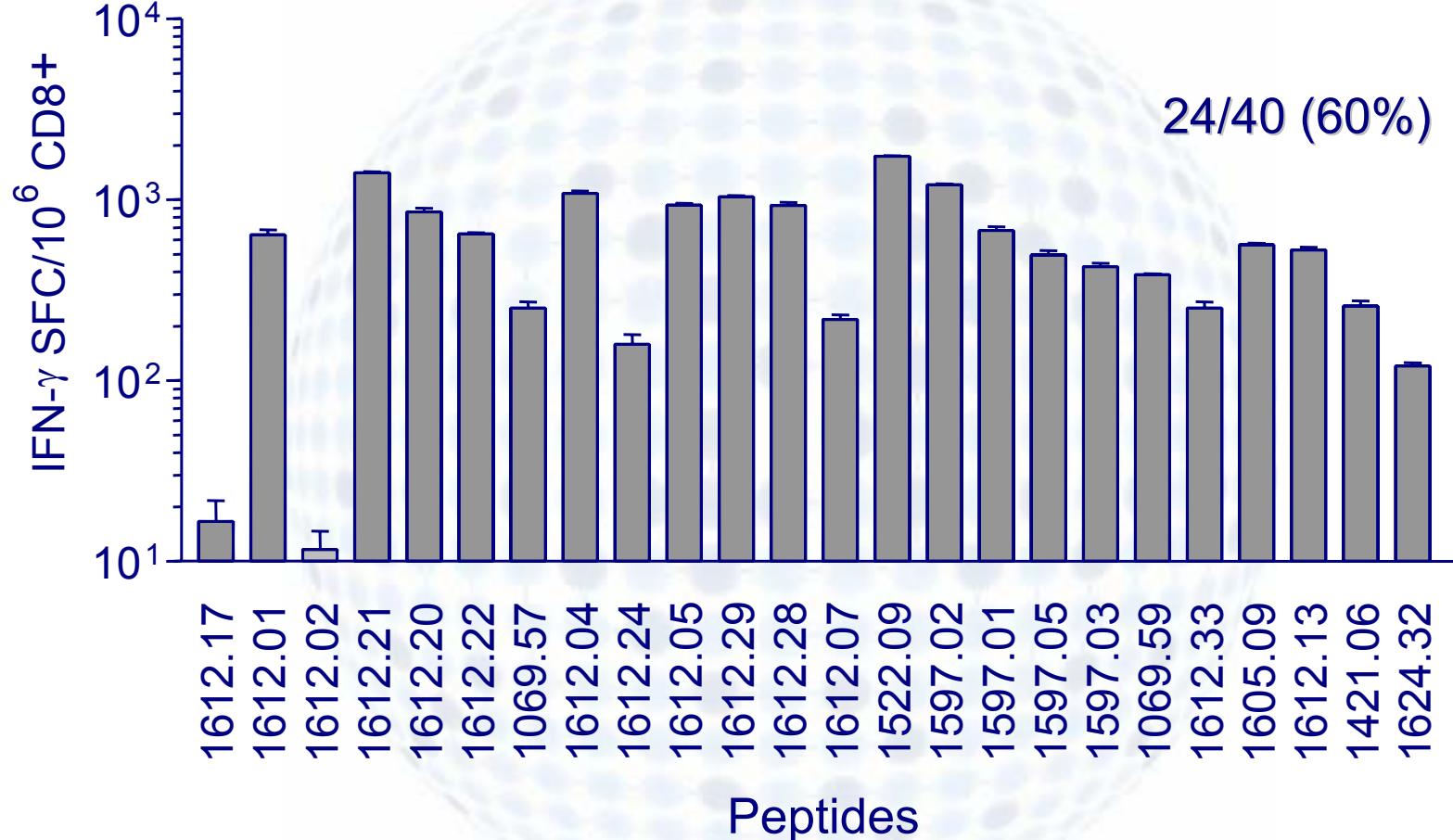
Breeding of HLA-A*2402 Founder Mice



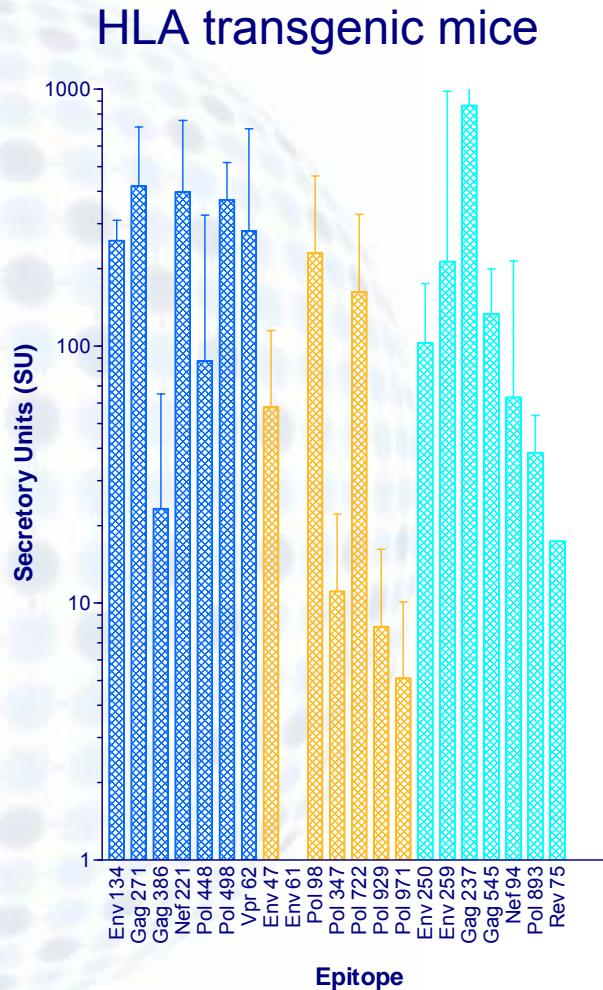
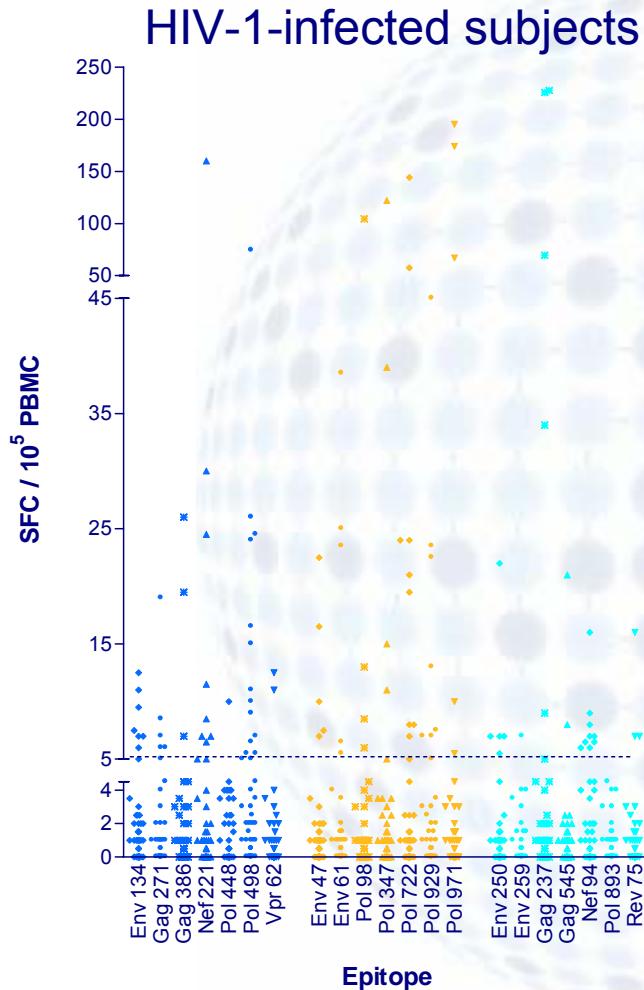
Validation Using Immune Responses to Known HLA-A24 Epitopes



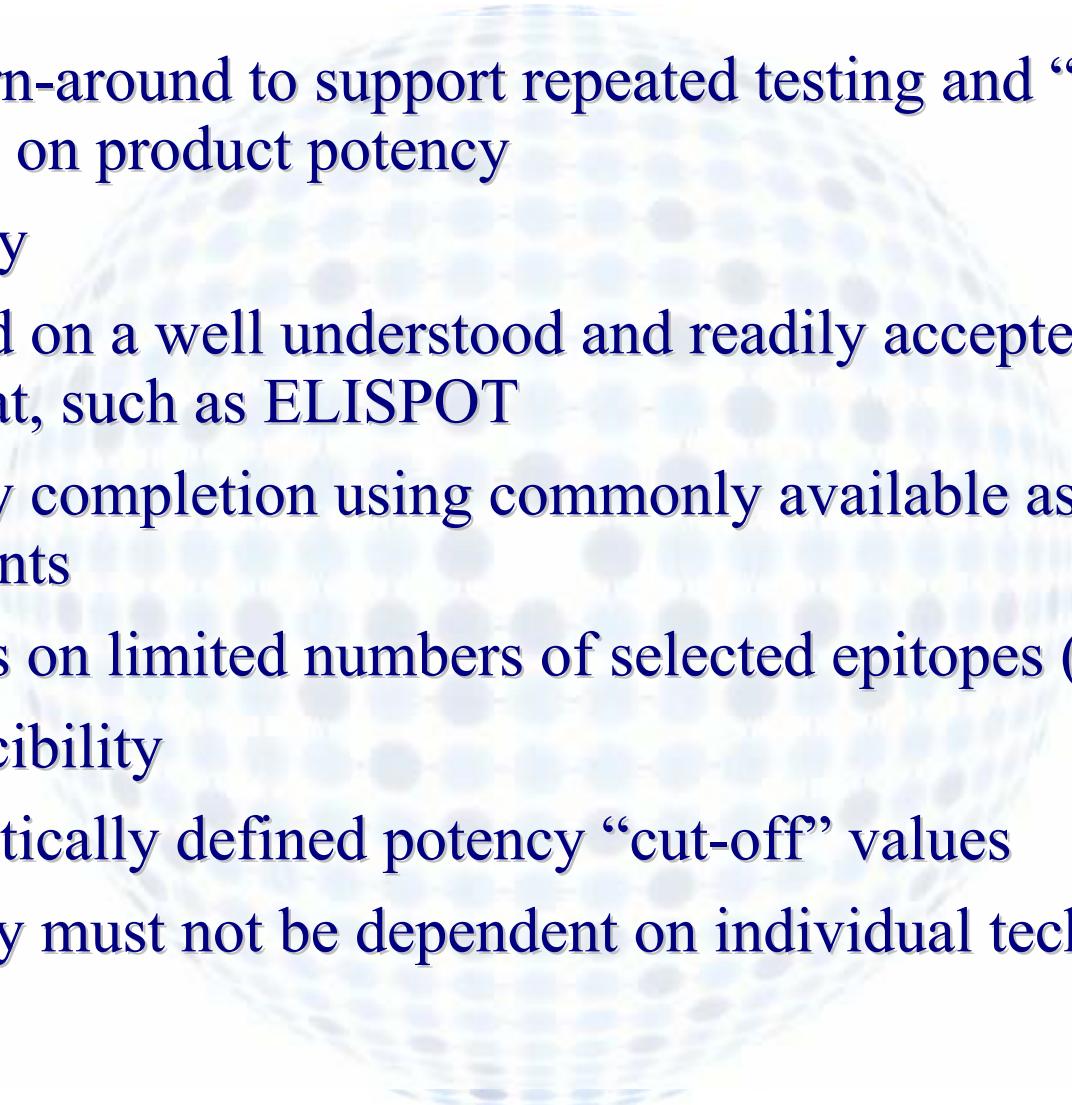
HIV Epitope Recognition Rate Using HLA-A24 Transgenic Mice



Similar Recognition of CTL Epitopes by HLA-2, -A11 and -B7 Transgenic Mice

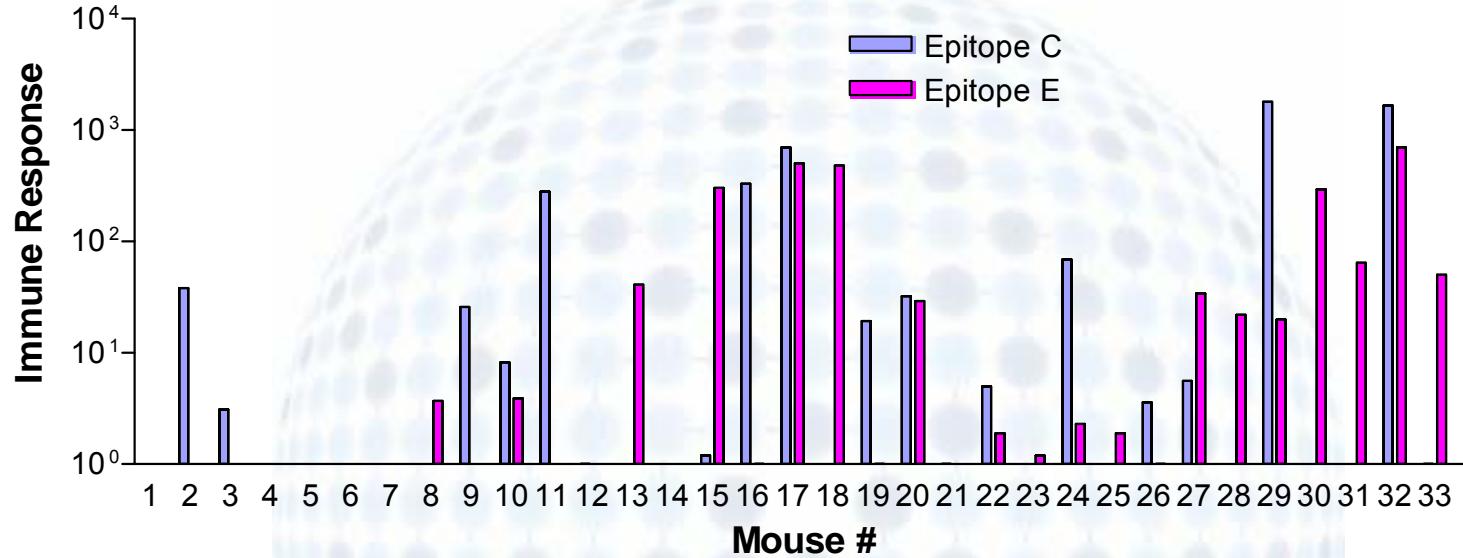


Potency Assay Requirements



- Rapid turn-around to support repeated testing and “real-time” decisions on product potency
- Simplicity
 - Based on a well understood and readily accepted assay format, such as ELISPOT
 - Assay completion using commonly available assay reagents
 - Focus on limited numbers of selected epitopes (1-2)
- Reproducibility
 - Statistically defined potency “cut-off” values
 - Utility must not be dependent on individual technical staff

Single Mouse Experiments Document Significant Assay Variation



- Immune responses were measured in 33 individual EP-HIV-1090 immunized mice
 - Single immunizations
 - ELISPOT

Use of Splenocyte “Pools” from Multiple Mice to Address Response Variation

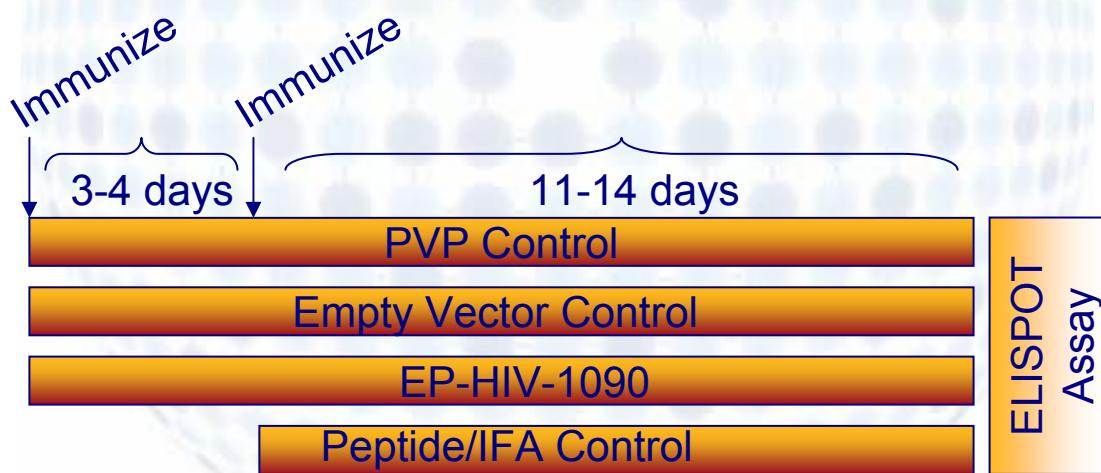
Epitope	Assay										Geomean SFC	Geomean/2sd
	1	2	3	4	5	6	7	8	9	10		
A	■			■	■	■	■			■	■	140
B	■	■	■	■	■	■				■	■	17.4
C		■	■	■	■	■	■	■	■	■	■	
D	■	■	■	■	■	■	■	■	■	■	■	261
E	■	■	■	■	■	■			■	■	■	20.9
F		■	■		■	■				■	■	

■ SPC/ 10^6 >10 and $p \leq 0.05$

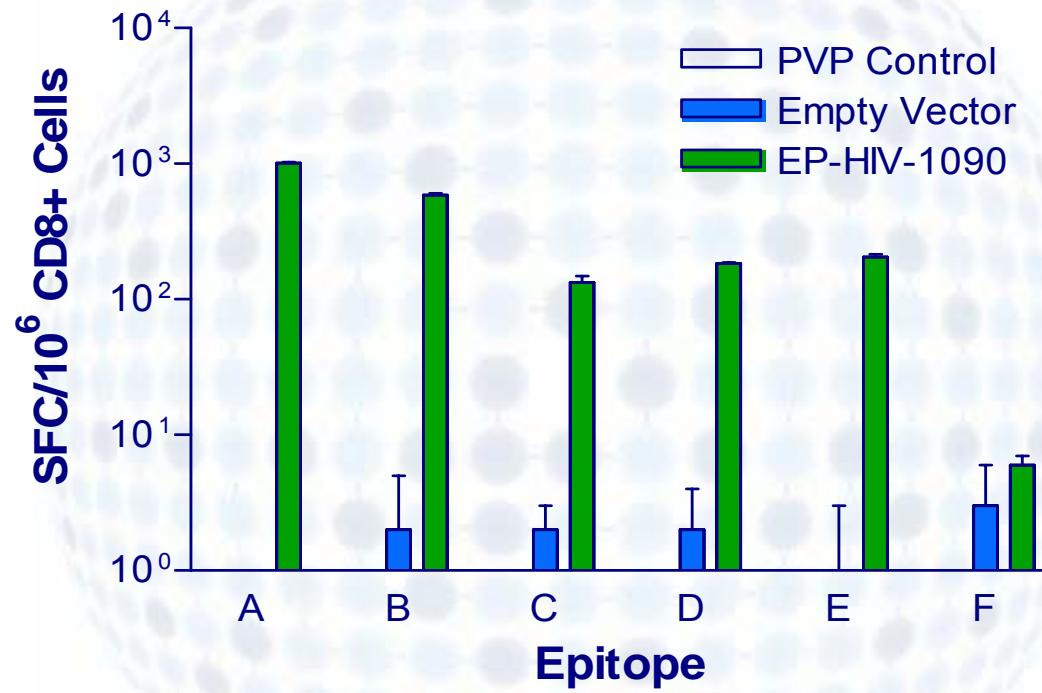
- Using splenocyte pools from ≥ 3 mice, induce positive responses more consistently
- Use of 5-6 mice/group results in reproducible induction of responses to all epitopes

Enhanced Immunization Strategy

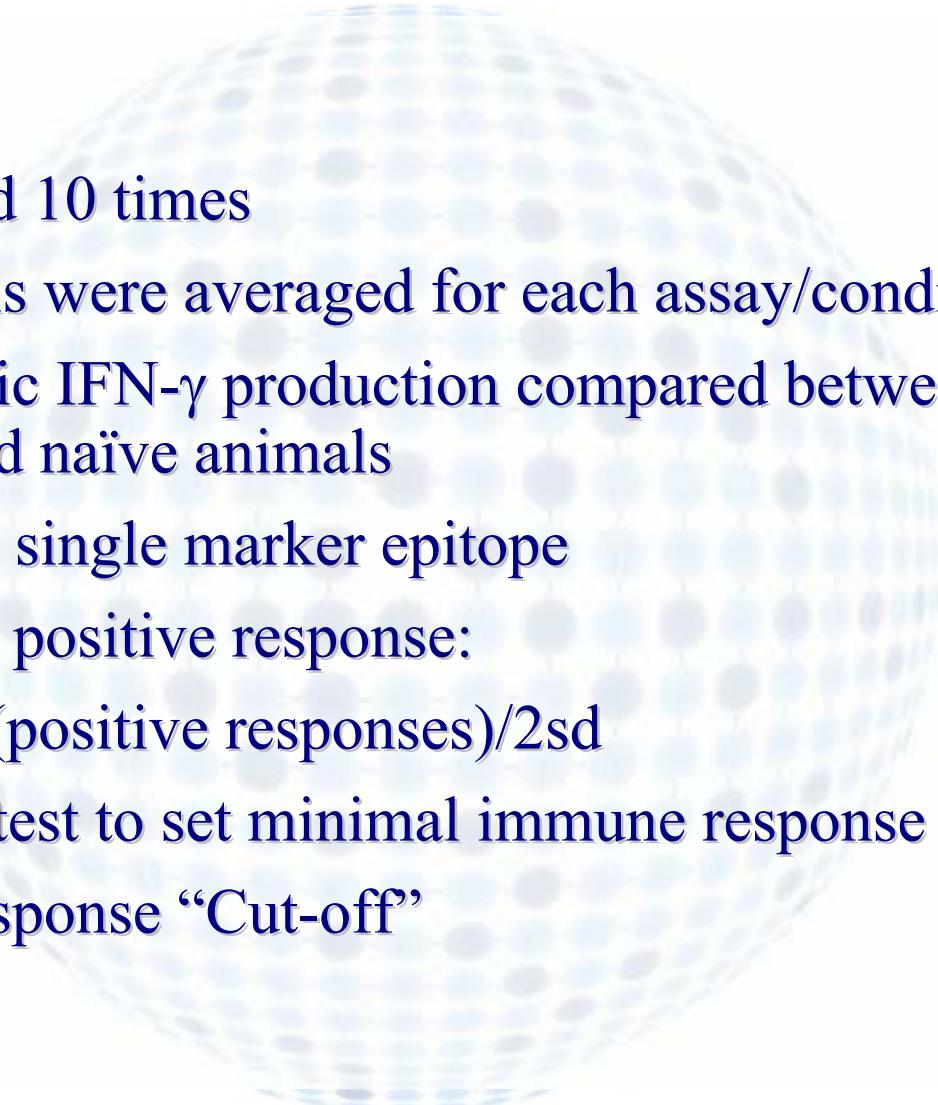
- DNA
 - Formulated in PVP
 - 50 µg / dose; 100 µg / mouse
- No site-of-injection muscle pre-treatment (Cardiotoxin)
- Two immunizations, 3-4 days apart



Representative Immune Responses

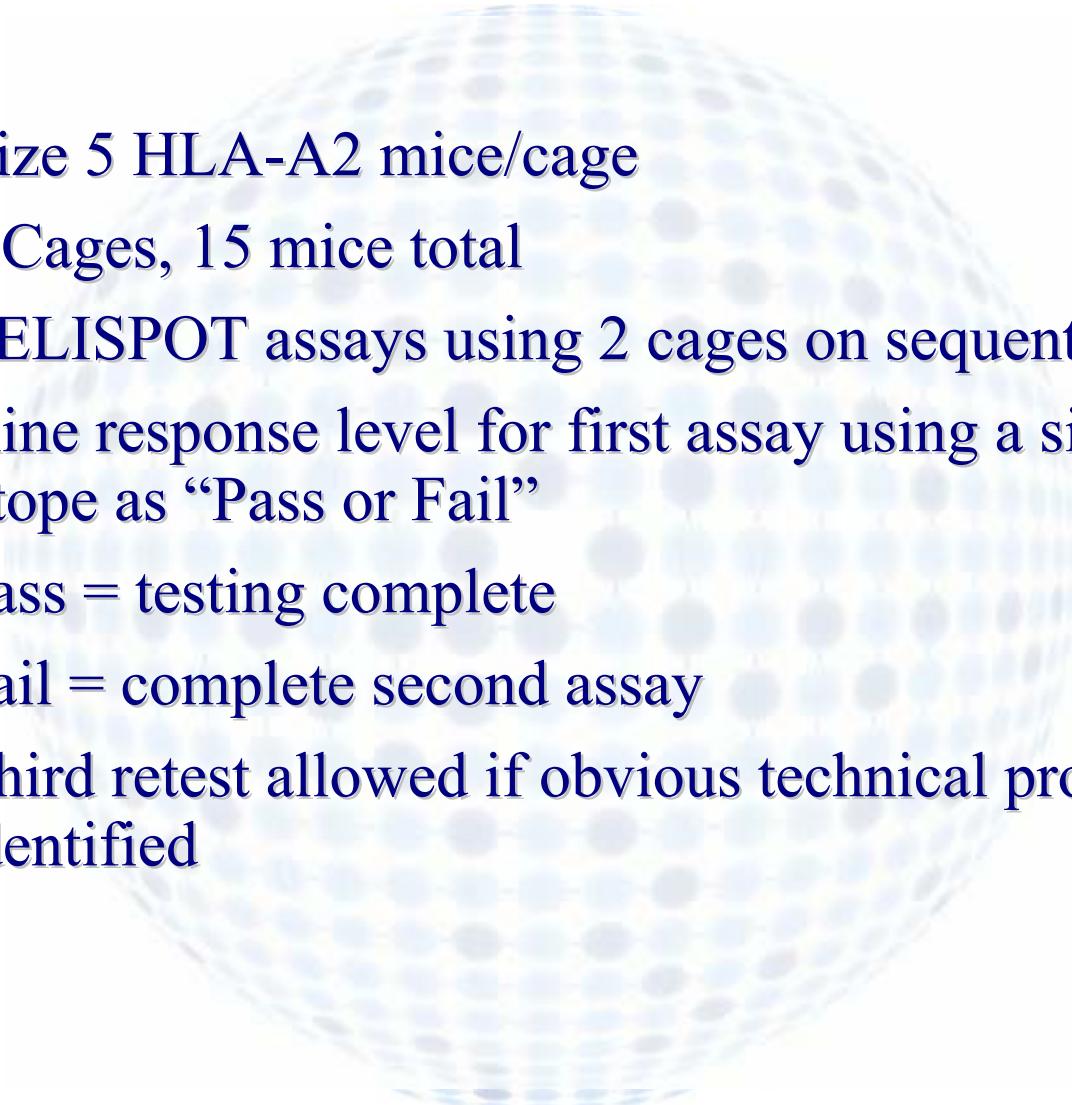


Potency Assay (ELISPOT) Validation

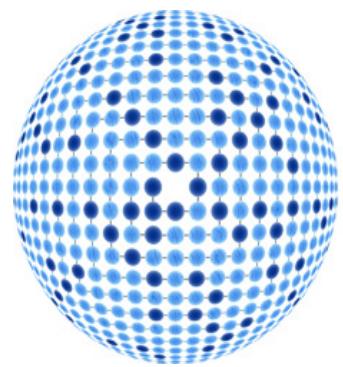


- 5 mice/group
- Assay repeated 10 times
- Triplicate wells were averaged for each assay/condition
- Peptide-specific IFN- γ production compared between immunized and naïve animals
- Select suitable single marker epitope
- Set criteria for positive response:
 - Geomean (positive responses)/2sd
 - 1-tailed T test to set minimal immune response level
 - Response “Cut-off”

Current Generation Potency Assay



- Immunize 5 HLA-A2 mice/cage
 - 3 Cages, 15 mice total
- Set-up ELISPOT assays using 2 cages on sequential days
- Determine response level for first assay using a single marker epitope as “Pass or Fail”
 - Pass = testing complete
 - Fail = complete second assay
 - Third retest allowed if obvious technical problem is identified



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